Claims

5

35

WO 2005/089933

A process for covalently binding a tagged protein to a polymer particle comprising:

contacting a tagged protein with a chelating agentpolymer particle conjugate wherein said tag comprises at least two histidine residues and at least two lysine residues and said chelating agent is tridentate, tetradentate or pentadentate and comprises at least two carboxyl groups and is coordinated by a metal ion, to form 10 a protein-polymer particle-chelating agent metal ion complex:

contacting said complex with a carbodiimide; and optionally

removing the metal ions. 15

- A process as claimed in claim 1 wherein said tag is a 2. HAT-tag.
- A process as claimed in claim 1 or 2 wherein said 20 carbodiimide is dicyclohexylcarbodiimide or N-(3dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) or a salt thereof.
- A process as claimed in any one of claims 1 to 3 25 wherein said chelating ligand comprises three carboxyl groups.
- A process as claimed in any one of claims 1 to 4 wherein said chelating ligand is tetradentate. 30
 - A process as claimed in any one of claims 1 to 5 6. wherein said chelating ligand is iminodiacetic acid, nitrilo triacetic acid, tris(carboxymethylethylene diamine or Cm-Asp.
 - A process as claimed in claim 6 wherein said 7.

WO 2005/089933 PCT/GB2005/000991

26

chelating ligand is Cm-Asp.

8. A process as claimed in any one of claims 1 to 7 wherein said polymer particle is magnetic.

5

- 9. A process as claimed in any one of claims 1 to 8 wherein said polymer particle is porous.
- 10. A process as claimed in any one of claims 1 to 9
 wherein said polymer particle has a diameter in the range
 0.2 to 1.5 microns.
 - 11. A process as claimed in any one of claims 1 to 10 wherein said polymer particle is a Dynabead.

15

- 12. A process as claimed in any one of claims 1 to 11 wherein said metal ion is a transition metal ion.
- 13. A process as claimed in claim 12 wherein said metal 20 ion is in the 2+ oxidation state.
 - 14. A process as claimed in claim 13 wherein said metal ion is Co^{2+} .
- 25 15. A process as claimed in any one of claims 1 to 14 wherein said metal ions are removed.
 - 16. A process as claimed in any one of claims 1 to 15 wherein said process is for isolating a tagged protein from a cell lysate.
 - 17. A tagged protein covalently bound to a polymer particle through the tag obtainable by a process as claimed in any one of claims 1 to 16.

35

30

18. A polymer particle covalently bound to a tagged protein via a linker comprising a residue of formula

WO 2005/089933

said tag comprising at least two histidine residues and at least two lysine residues.

27

- 19. A magnetic polymer particle covalently bound to a protein via a tag on said protein, said tag comprising at least two histidine residues and at least two lysine residues, said particle comprising a linking group which binds to said tag via said at two least lysine residues through amide linkages.
- 20. A plurality of magnetic particles as claimed in claim 15 19 said particles being monodisperse.
 - 21. An assay comprising a process as claimed in any one of claims 1 to 16.
- 20 22. Use of a magnetic polymer particle covalently bound to HAT-tagged protein in an assay.